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Immunohistochemical expression of MMP-9, TIMP-2, E-cadherin and vimentin in ameloblastomas and their implication in the local aggressive behavior of these tumors

ALMA FLORESCU[1)], CL. MĂRGĂRITESCU[2)], CRISTIANA EUGENIA SIMIONESCU[2)], A. STEPAN[2)]

[1)]*PhD student*
[2)]*Department of Pathology*
University of Medicine and Pharmacy of Craiova

Abstract

Among the benign epithelial odontogenic tumors, the ameloblastoma occupies a special place because of their local aggressive behavior with devastating jaw destructions, which lead to a high recurrence rate, even following the radical surgery. In an attempt to clarify the mechanisms underlying this behavior we immunohistochemically investigated the reactivity of different histological variant of ameloblastoma to MMP-9, TIMP-2, E-cadherin and vimentin. A semiquantitative assessment of their reactivity in the epithelial neoplastic compartment was done and statistical correlation was attempt with histological variant and between them. The MMP-9 and TIMP-2 reactivity was observed with variable intensity both in the neoplastic epithelium and in the stroma that surround the ameloblastic proliferations. Only for MMP-9, the statistical tests proved the existence of significant differences within major ameloblastic histological variants, with the highest reactivity acanthomatous type. The stroma independent of the histological variant had the highest reactivity at the invasive front adjacent to the tumoral islands. Immunoreactivity for E-cadherin was more obvious in the follicular type at the level of stellate-reticulum like cells, and decreased in the peripheral columnar cells, as they are closer to the invasion front. Vimentin reactivity was present in the neoplastic epithelium only in the peripheral columnar cells at the invasion front but at this site, the stroma had the highest expression. Statistical analysis proved the existence of inverse relationship between MMP-9 and E-cadherin scores, while vimentin score had an inverse relationship both with TIMP-2 and E-cadherin scores. Our results proved the implication of these four markers in the growth of ameloblastomas and as a consequence their utility in monitoring of local aggressiveness of these tumors.

Keywords: ameloblastoma, immunoreactivity, MMP-8, TIMP-2, E-cadherin, vimentin.

Introduction

Solid ameloblastoma is a slow growing odontogenic epithelial tumor of jaw, local invasiveness and that can recur despite adequate surgical removal. It is a relatively rare dental tumor accounting for less than 1% of all tumors and cysts arising in jaw bones [1]. It seems, that after odontomas is the second most common odontogenic neoplasm with a peak incidence is in the 3rd to 4th decades of life and has an equal sex distribution [2–4]. Most of solid ameloblastomas in the molar-ramus area of the mandible, and are occasionally associated with unerupted third molar teeth [2, 5].

Generally, it is considered that these tumors develop from odontogenic the remnants of the dental lamina cells of the dental organ, or can sometimes arise because of neoplastic changes in the lining or the wall of a non-neoplastic odontogenic cyst, most commonly the dentigerous cysts and odontogenic keratocysts [6].

In the 2005, the *WHO* classification of head and neck tumors divided the benign ameloblastoma into four variants: solid/multicystic, extra-osseous/peripheral, desmoplastic, and unicystic [2]. The solid/multicystic ameloblastoma is the most common variety (accounting for 86% of all cases) and histopathologically can be divided into a follicular and a plexiform type; the follicular type can be further subdivided into a spindle cell type, an acanthomatous type, a granular type and a basal cell type [2].

Although characterized as a benign neoplasm, solid ameloblastoma is a locally invasive, highly destructive tumor of the jaws, with a high recurrence rate even following radical surgery [7]. This local biological behavior of solid multicystic ameloblastoma is an argument for including it in the group of low-grade malignant tumors [8]. The mechanisms underlying the local invasiveness of this neoplasm have yet to be clarified.

Thus, identification of prognostic markers of the ameloblastoma's biologic behavior is of considerable importance to determine the most appropriate therapeutic approach and establish the prognosis of patient. In respect to this, our study aims to investigate immuno-histochemical the E-cadherin, MMP-9, TIMP2 and vimentin reactivity in 17 solid ameloblastomas.

Materials and Methods

The selected sample at the Laboratory of Pathology, Emergency County Hospital of Craiova, consisted of 17 cases of solid ameloblastomas, selected within 15 years from 1996–2011. Histological sections of 4-μm thickness were cut from paraffin archived blocks and Hematoxylin–Eosin stains. Then they were microscopically evaluated by two-experienced pathologists and diagnosed according to the classification of the *World Health Organization* [2]. In Table 1 are recorded the histopathological features of these tumors and also the main clinico-epidemiological data reviewed from the medical records of these patients (Table 1).

Table 1 – *Clinico-morphological parameters of the ameloblastomas*

Clinico-morphological parameters		No. of cases	%
Age [years]	20–40	10	58.9
	40–60	5	29.4
	>60	2	11.7
Sex	Female	6	35.3
	Male	11	64.7
Tumor topography	Mandible	15	88.2
	Maxillary	2	11.8
Tumor extension	Bone	2	11.7
	Skin	1	5.9
Histopathological variants	Typical follicular ameloblastoma	12	70.6
	Acanthomatous ameloblastoma	3	17.6
	Granular cell ameloblastoma	2	11.8

Immunohistochemical processing

From the paraffin-embedded material were cut 4-μm thick sections that were mounted on glass slides coated with poly-L-Lysine. Next, the sections were deparaffinized in xylene, dehydrated in ethanol, and immersed in distillated water containing 3% hydrogen peroxide for 30 minutes to block endogenous peroxidase activity. Then we made antigen unmasking by 20 minutes heat induced epitope retrieval in 10 mmol/L citrate buffer, pH 6. Subsequently, the unspecific binding sites were blocked with 5% BSA/PBS for one hour. As immunohistochemical technique we used a standard Streptavidin–Biotin peroxidase system. Next, the sections were submitted to the Streptavidin–Biotin method using the following primary monoclonal mouse anti-human antibodies: MMP-9 (clone 2C3, Santa Cruz Biotechnology, Redox, Romania – code sc-21733), TIMP-2 (clone 3A4, Santa Cruz Biotechnology, Redox, Romania – code sc-21735), E-cadherin (clone NCH-38, Dako, Redox, Romania – code M3612), and vimentin (clone V9, Dako, Redox, Romania – code M0725). All these antibodies were diluted 1:100 and the slides were incubated overnight at 4^0C with these antibodies diluted 1:100. The reactions were amplified with LSAB2 (Dako, Redox, Romania – code K0675) and visualized with 3,3'-diaminobenzidine (DAB) (Dako, Redox, Romania – code K3468). For counterstaining, we used Mayer's Hematoxylin. Negative-control stainings were done by omitting the primary antibodies.

Immunostaining assessment

Immunohistochemical staining was evaluated using descriptive and semiquantitative methods based on an adaptation of the criteria of Alves Pereira KM *et al.* (2010) [9]. Each case was examined and scored separately by three independent observers (FA, MC, SA) with a minimal interobserver variability (< 5%). Only the neoplastic cells were evaluated, but without considering differences in intensity of immunoreactivity. The cellular location of immunostaining for MMP-9, TIMP-2 and vimentin was determined in cytoplasm, while for E-cadherin was determined in membrane and cytoplasm.

In brief, the stained sections were screened at a magnification of ×400 under a Nikon Eclipse 55i microscope (Nikon, Apidrag, Bucharest) equipped with a 5-megapixel cooled CCD camera and the Image ProPlus AMS7 software (Media Cybernetics Inc., Buckinghamshire, UK) to identify the regions with the highest staining intensity. A total of 1000 cells were counted in these areas, and the average numbers of positive cells were recorded. Semiquantitative analysis of immunostained cells was performed using the following scores: 0 (without any reactivity in the parenchyma compartment), 1 (<10% of positive cells), and 2 (>10% of positive cells).

After obtaining the data, a descriptive analysis of the results was carried out. In the statistical analysis, the non-parametric Pearson's *chi*-square test, with a significance level of 5%, was used to evaluate the difference between the scores in each tumor. Their correlation with main clinico-epidemiological data was evaluated.

Results

Clinico-morphological data

The mean age of the patients was 39.2 years with the youngest of 27 years and the oldest of 71 years. In term of gender, male outnumbered females by a 1.83:1 ratio. Most cases (88.23%) involved the mandible with molar-ramus area as the most commonly affected. In two cases, tumors invaded the jaw bone and in another one the neoplastic extension was done into skin. Histopathologically, all investigated cases were solid ameloblastoma with typical follicular growth pattern in 12 cases, acanthomatous in three cases or with granular cells in two cases.

MMP-9 immunoreactivity

The immunoexpression of MMP-9 was detected only in 13 of 17 cases (76.5%). The immunoreactivity for MMP-9 was evident both in parenchymal and stromal cells with a predominantly diffuse pattern. In the parenchyma MMP-9 reactivity were mostly located in the cytoplasm of the ameloblastic-like columnar cells and stellate-reticulum like cells (Figure 1, A and B). In term of histopathological variant, the greater intensity of MMP-9 was noticed in the acanthomatous type and |at the level of squamous metaplasia areas from the follicular ameloblastoma (Figure 1C). The lowest MMP-9 reactivity was recorded in granular cell ameloblastoma, one case being negative (Figure 1D). In the stromal

compartment, MMP-9 staining was observed in fibroblasts, endothelial and inflammatory cells in all cases studied with predominant reactivity around tumor cell islands or nests (Figure 1, E and F).

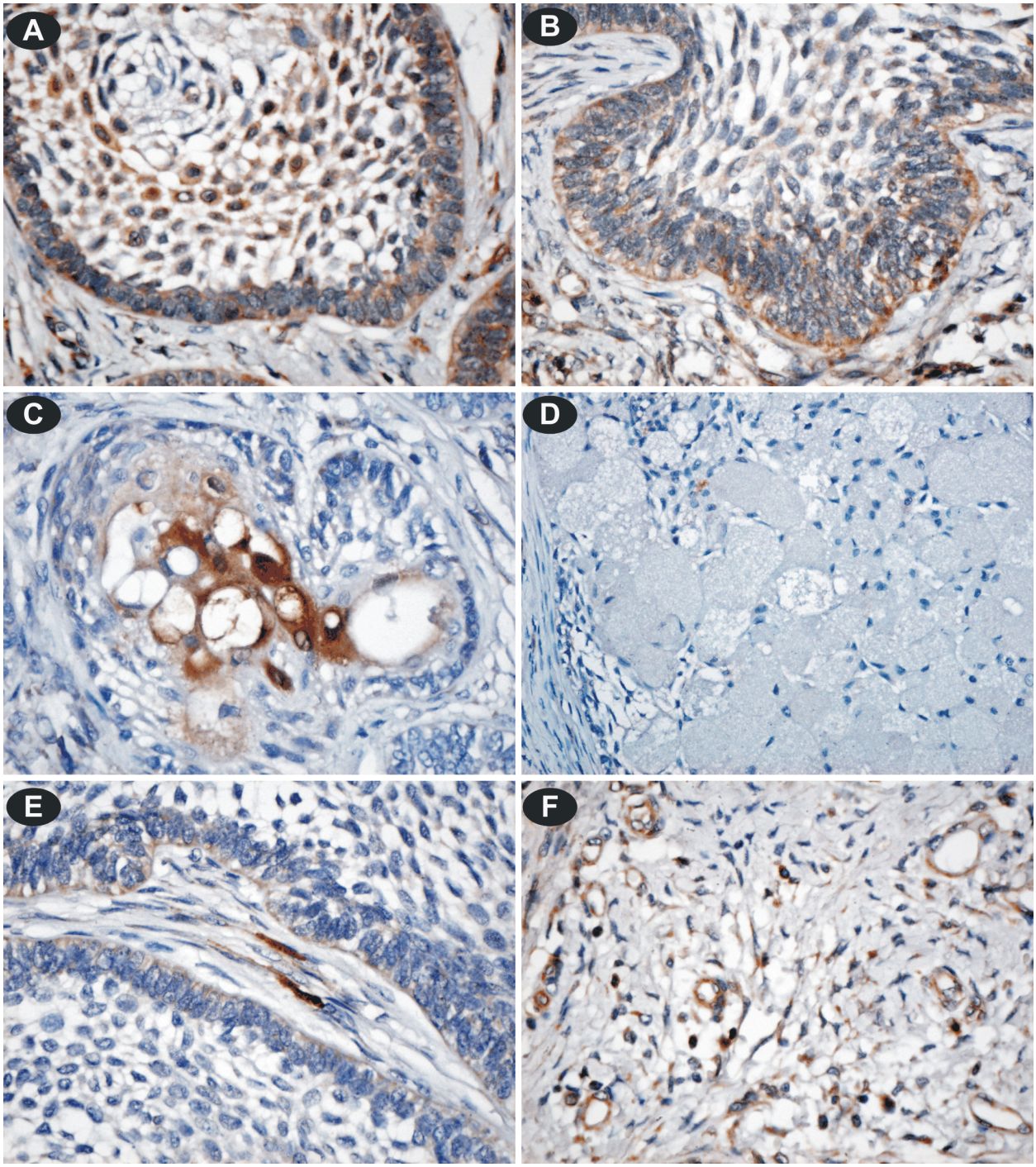

Figure 1 – ***MMP-9 reactivity in ameloblastomas: (A and B) Folicullar ameloblastoma positive reaction in the cytoplasm of columnar cells and stellate-reticulum like cells, ×400; (C) Acanthomatous ameloblastoma positive reaction in the acanthomathous area, ×400; (D) Granular cell ameloblastoma negative reaction in the granular cells, ×400; (E and F) Follicular ameloblastoma, positive reaction in cytoplasm of fibroblasts, endothelial and inflammatory cells, ×400.***

The semiquantitative MMP-9 analyze proved that the majority of investigated cases had score 1 (47%), followed by the cases with score 2 (29.5%). Histopathologically, the follicular type had the lowest reactivity, score 2 being observed only in one case. The highest reactivity was noticed in acanthomatous type, all three investigated cases had score 2 (Table 2). These differences were also highlighted by statistical tests $\{\chi^2(4, N=17)=10.5, p=0.032\}$ (Figure 2A).

TIMP-2 immunoreactivity

The reactivity to this marker was present in all investigated cases both in the neoplastic epithelium and stroma. In the ameloblastoma parenchyma the TIMP-2 reactivity was found with different intensity both in the periferical cuboidal/columnar cells and stellate-reticulum like cells (Figure 3A). In the follicular type the most intense reaction was observed in peripheral cells, especially at the basal pole toward basal

membrane and also at the level of squamous metaplasia areas (Figure 3B). Histopathologically, the reactivity was more obvious in acanthomatous type (Figure 3C) than in the follicular or granular cell types (Figure 3D). In the stromal compartment, the TIMP-2 reactivity was observed in fibroblasts, endothelial cells, and in inflammatory in all cases studied (Figure 3, E and F).

The semiquantitative TIMP-2 analyze showed that in the neoplastic epithelial component regardless of histological type the prevalent score was 2 achieved in nine (53%) of cases. In term of histopathological variant the granular cell type followed by follicular type had the lowest TIMP-2 scores (Table 2). Statistical analysis did not reveal any significant differences between TIMP-2 scores of immunoreactivity and histological subtype $\{\chi^2(2, N=17)=2.55, p=0.279\}$.

Table 2 – ***Immunoreactivity for MMP-9, TIMP-2, E-cadherin, and vimentin in ameloblastomas***

	Score assessment of the immunoreactions			
Ameloblastoma type	***MMP-9***	***TIMP-2***	***E-cadherin***	***Vimentin***
Follicular	0*	1	2	0
Follicular	1	2	2	0
Follicular	1	1	1	1
Follicular	1	1	2	0
Follicular	2	1	1	1
Follicular	0	2	1	0
Follicular	1	2	2	0
Follicular	1	2	2	0
Follicular	0	2	2	0
Follicular	0	1	2	0
Follicular	1	1	1	1
Follicular	1	2	2	0
Acanthomathous	2	2	1	0
Acanthomathous	2	2	1	0
Acanthomathous	2	2	1	0
Granular cell	0	2	2	0
Granular cell	1	1	2	0

*Scores: 0 – No positive reaction; 1 – <10% positive neoplastic cells; 2 – >10% positive neoplastic cells.

E-cadherin immunoreactivity

Immunoreactivity was restricted to the neoplastic epithelial component of solid ameloblastoma and was present in all investigated cases. The most intense reactivity was observed in the stellate-reticulum like cells and central angular or polyhedral cells (Figure 4A). The intensity deacreased in the peripheral columnar cells, especially to the invasive front (Figure 4B). The pattern of E-cadherin reactivity was predominantly on the membrane at cell–cell boundaries, but a cytoplasmic reaction was also noticed in the peripheral columnar cells. The E-cadherin expression was weak or even lost in the squamous metaplastic areas (Figure 4C) and keratinized areas of acanthomatous ameloblastomas, and was reduced in the granular cell clusters of the granular cell ameloblastomas.

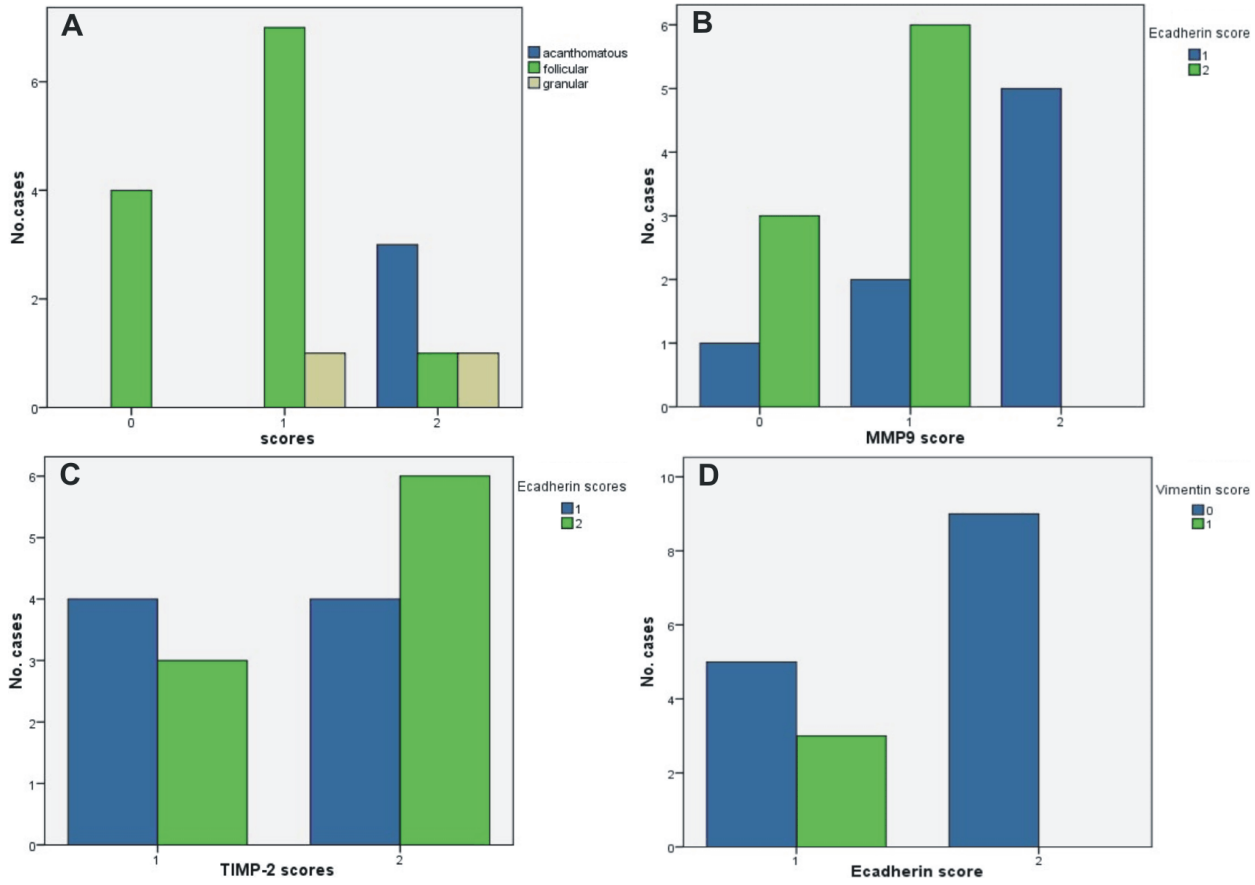

Figure 2 – ***Pearson test: (A) Direct relationship between MMP-9 score and histological variants of ameloblastoma; (B) Inverse relationship between MMP-9 and E-cadherin scores. (C) Inverse relationship between TIMP-2 and vimentin scores; (D) Inverse relationship between E-cadherin and vimentin scores.***

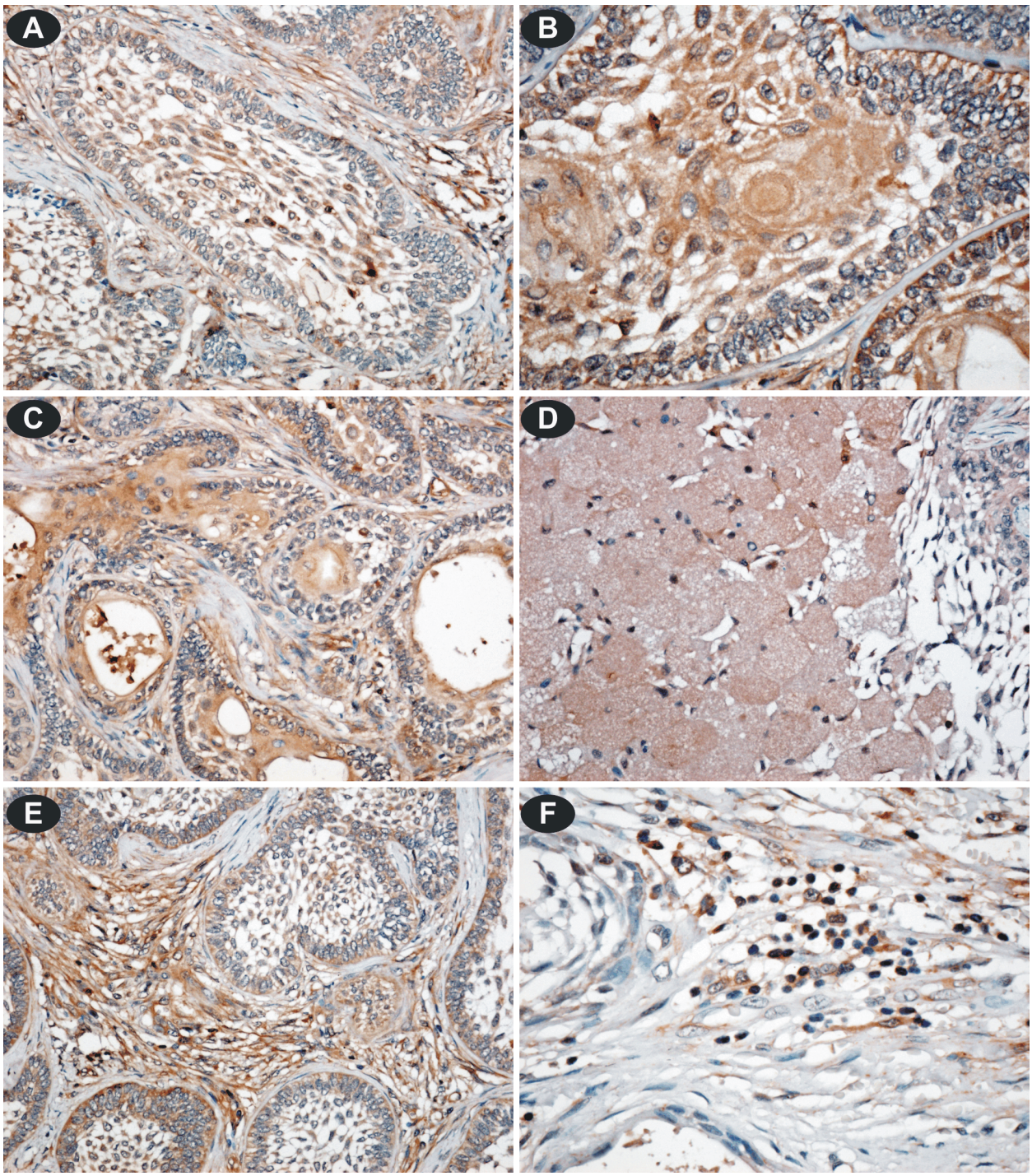

Figure 3 – ***TIMP-2 reactivity in ameloblastomas: (A) Folicullar ameloblastoma positive reaction in the cytoplasm of columnar cells and stellate-reticulum like cells, ×400; (B) Folicullar ameloblastoma positive reaction in the squamous metaplasia areas, ×400; (C) Acanthomatous ameloblastoma positive reaction in the acanthomathous area, ×400; (D) Granular cell ameloblastoma weak cytoplasmic reaction in the granular cells, ×400; (E and F) Follicular ameloblastoma, positive reaction in cytoplasm of fibroblasts, endothelial and inflammatory cells, ×400.***

The immunohistochemical assessment revealed that the most prevalent score was 2, observed in 53% of cases, especially in follicular type ameloblastomas (eight from the twelve cases). In the granular cell variant (one of the two cases) and especially in the acanthomatous type (all three cases) the percentage of immunostained cells was <10% (score 1) (Table 2). Statistical analysis did not reveal any significant differences between E-cadherin scores of immunoreactivity and histological subtype $\{\chi^2(2, N=17)=4.28, p=0.117\}$.

Vimentin immunoreactivity

The vimentin reactivity was obvious in the stromal compartments of the investigated ameloblastomas. The pattern of the reaction was a cytoplasmic one. The strongest vimentin expression in stromal cells was found in those cases that had bone (two cases) and skin extension (one case). All these three cases were histopathologically of follicular type. As tumoral topography, the strongest reaction was observed in the stromal cells adjacent to the invasive front (Figure 4D). Also

examining serial sections that were stained for vimentin and E-cadherin we had noticed that those stromal cells that had highest vimentin reactivity were closed to the peripheral columnar/cuboidal cells from the invasive neoplastic islands that had the lowest E-cadherin expression (Figure 4E). Intratumoral the stromal reactivity was variable; in some cases, the vimentin reactivity was missing (Figure 4F).

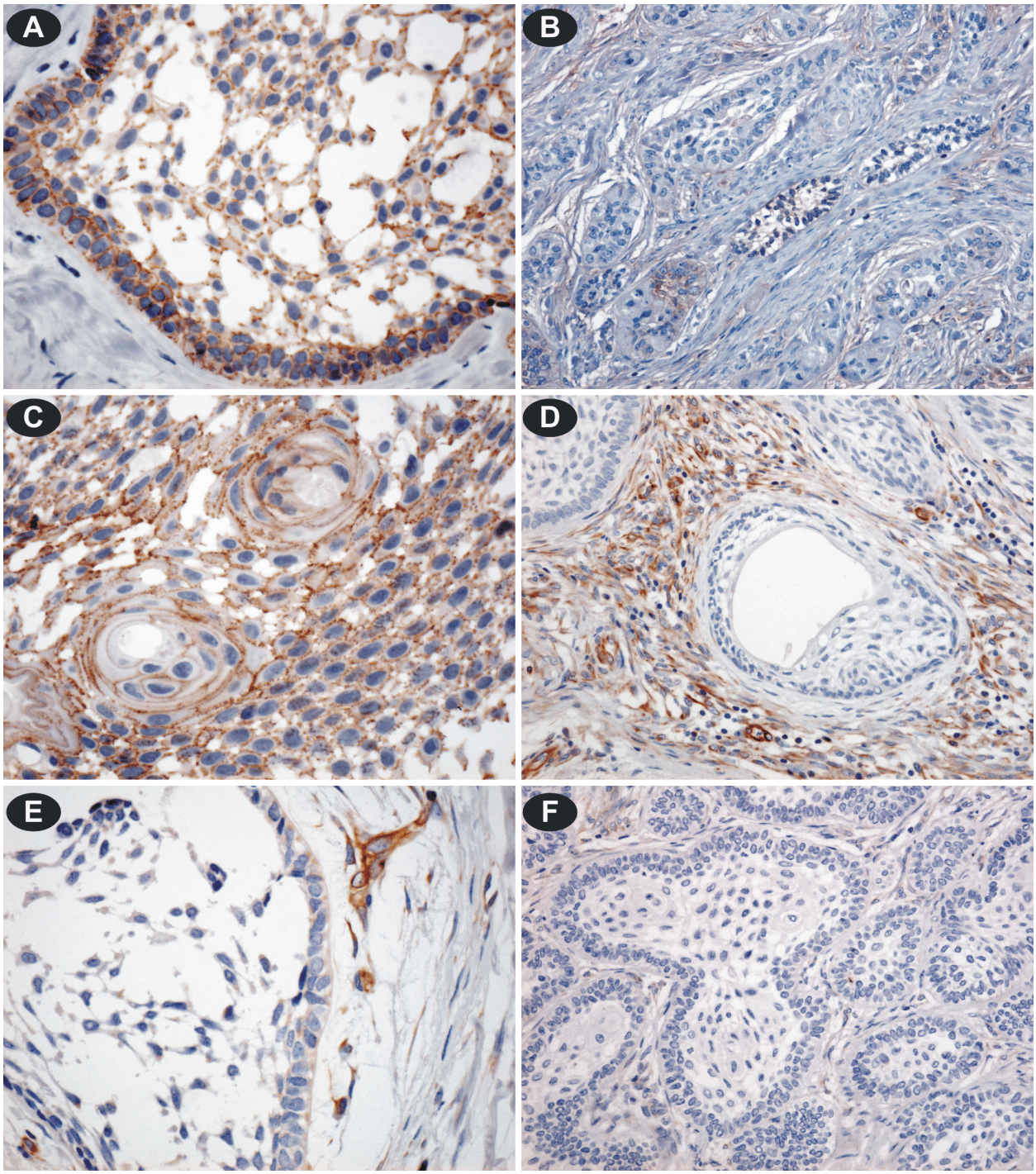

Figure 4 – ***E-cadherin reactivity in ameloblastomas: (A) Folicullar ameloblastoma positive reaction in the cytoplasm of columnar cells and stellate-reticulum like cells, ×400; (B) Folicullar ameloblastoma positive reaction in the squamous metaplasia areas, ×200; (C) Acanthomatous ameloblastoma positive reaction in the acanthomathous area, ×400. Vimentin reactivity in ameloblastomas: (D) Follicular ameloblastoma, positive reaction in cytoplasm of stromal cells adjacent to the invasive front, ×200; (E) Follicular ameloblastoma, weak positive reaction in cytoplasm of columnar cells from the periphery of tumoral islands and strong reaction in adjacent stromal cells, ×400; (F) Follicular ameloblastoma, negative both in epithelial and stromal compartments, ×200.***

Generally speaking, the neoplastic epithelial compartment had weak vimentin reactivity or no reaction was observed in the neoplastic cells. This reactivity was not influenced by the histological type. The neoplastic vimentin reaction was observed in the peripheral columnar/cuboidal cells from the invasive neoplastic islands, especially in those cases with bone or skin extension. The semiquantitative assessment of the neoplastic vimentin reactivity was extremely difficult to quantify. The number of positive neoplastic cells was

less than 1% and that appeared only in those three cases of follicular ameloblastoma that presented an invasion in the surrounding tissues. The statistical analysis did not reveal any significant differences between the vimentin scores of immunoreactivity and the histological subtype $\{\chi^2(2, N=17)=1.51, p=0.468\}$.

When we investigate the statistical correlations between the immunoreactivity scores in the ameloblastic cells for these four markers we found that:

- between MMP-9 and E-cadherin scores was a inverse relationship $\{\chi^2(2, N=17)=7.96, p=0.019\}$, (Figure 2B);
- between TIMP-2 and vimentin scores was a inverse relationship $\{\chi^2(1, N=17)=2.55, p=0.279\}$, (Figure 2C);
- between E-cadherin and vimentin scores was a inverse relationship $\{\chi^2(1, N=17)=4.09, p=0.043\}$, (Figure 2D).

Discussion

The main biological feature of the ameloblastoma is its locally invasive behavior that is responsible for the higher postoperative recurrence rate, even following the radical surgery. Both its pathogenesis and invasive growth remains incompletely understood.

The invasion means the penetration by tumor cells into the neighboring territories and their occupation [10], and involves the interactions of the tumor cells with the extracellular matrix and basement membranes [10, 11]. The invasion is not a process specific to cancer; it is also present during embryonic development, in healthy organs, and in many non-cancerous diseases [11]. As major steps in this process are included adhesion, proteolysis of extracellular matrix component, reorganization of the cytoskeleton, and cell migration [12].

The extracellular matrix (ECM) is a dynamic reticulated structure present in between cell to cell, which play a key role in both normal and disease process as angiogenesis, inflammation, wound healing and tumor invasion [13]. Thus, it provides a physical barrier between cell and tissues, as well as a scaffold for cell growth, migration, differentiation and survival, and undergoes continuous remodeling [10]. The exact role of EMT in tumor progression is still debatable, but it is thought to be particularly important in cancers showing single-cell migration and early dissemination of tumor cells. In contrast, the invasion of large cell sheets into neighboring tissue, often called collective cell migration, is less well understood. These cell sheets maintain their expression of epithelial adhesion structures, but, nonetheless, invade into the surrounding tissue and thereby destroy the host organ [14]. In respect to these, our study aims to investigate the expression in solid ameloblastoma of some markers that are known to be involved in tumor invasion such as: MMP-9, TIMP-2, E-cadherin and vimentin.

Matrix metalloproteinases (MMPs) are capable of ECM remodeling, favoring the invasion and proliferation of neoplastic cells [15]. In addition, MMPs act on the non-matrix substrates, including cell-surface and matrix-bound growth regulators, releasing them from stores [16]. Activated MMPs are generally present in greater amounts in and around malignant tissues than in normal, benign, or premalignant tissues, with the highest expression occurring in areas of active invasion at the tumor–stroma interface [14]. The MMP subfamily of gelatinae, which includes gelatinae-A (MMP-2) and *gelatinae-B* (*MMP-9*), can cleave collagen IV and the structural components of basement membranes as a preface to invasion.

Our study revealed that the expression of MMP-9 was present both in the epithelial and stromal neoplastic compartments of the solid ameloblastoma. In the neoplastic epithelium, the MMP-9 reactivity was present in both peripheral columnar cells and stellate-reticulum like cells with the highest expression at the level of the squamous metaplasia areas. However, both the intensity and the overall rate of the MMP-9 positive neoplastic cells in the solid ameloblastomas were small. Instead this reactivity was far more evident in the stromal compartment, the highest expression being recorded around the islands from the invasion front, especially in those cases which extended into the surrounding tissues.

There have been numerous reports found regarding researches associated with the MMPs in ameloblastoma, many of them showing similar results to our study [17–23]. The presence of MMP-2 and -9 in the ameloblastomas may be related to the cell differentiation that occurs in tumor cells; columnar or cubic cells of the periphery of the nests of the odontogenic epithelium, recalling the ameloblasts, but also did not reach the necessary maturity to the formation of enamel [20, 24]. Also MMP-1, -2, and -9 was proved to be produced in the stromal cells of the ameloblastomas such as fibroblasts, endothelial cell, lymphocytes, plasma cells, macrophages, and neutrophils that also contribute to the ECM degradation and growing tumor [17, 18, 20]. Many authors suggested that the MMPs present in the ameloblastoma mediate bone resorption, which in turn release growth factors and cytokines entrapped within the bone matrix making its available to ameloblastoma cells located near the bone tissue [17, 18, 21, 22, 25]. Therefore, on the one hand, the ameloblastoma cells proliferation occurs and on the other hand, these neoplastic cells secrete more MMPs creating a "pre-invasive niche", which are related to the invasiveness of the ameloblastoma.

Tissue inhibitors of matrix metalloproteinases (TIMPs) are a family of four intrinsic inhibitors for MMPs, designated TIMP-1 to TIMP-4 [26]. The major function of TIMPs is to inhibit the active forms of MMPs; therefore, the balance between MMPs and TIMPs ultimately determines the extent of the ECM degradation in physiological and pathological conditions [27, 28].

Our study proved that *TIMP-2* was expressed both in the neoplastic epithelium and stroma with an intensity that was superior to MMP-9. The most intense reaction was observed in peripheral cells, especially at the basal pole towards the basal membrane. The statistic analyze did not found any correlation between the immuno-reactive TIMP-2 score and the histological types of ameloblastomas, even at the intensity level reactivity was more obvious in the follicular type. Also, we found

that the TIMP-2 score was inverse correlated with the vimentin score.

In the immunohistochemical study developed by Kumamoto H *et al.* (2003), it was noticed an increased expression of TIMP-1 and TIMP-2 related to weak immunoreactivity for MMP-9 [17]. Thus, the authors suggest that the TIMPs contribute to suppress the tumor progression in the ameloblastomas through the inhibition of MMP-9 activity. Also, Siqueira AS *et al.* (2010) observed an intense and diffuse TIMP-2 reactivity in ameloblastomas, which was associated with lower aggressiveness of the tumors [22]. Moreover, Wang A *et al.* (2008) showed that the overexpression of TIMP-2 in the ameloblastoma cells was associated with the suppression of MMP-2 activity and a consequently lower invasion potential [29]. Unlike them, Henriques ÁC *et al.* (2011) observed a lower expression of TIMP-2 that was responsible for a more aggressive behavior of this tumor compared with the other lesions studied, a fact supported by the higher expression of MMP-9 [30]. In addition, although the TIMP-2 is a tissue inhibitor of activated-MMP-2, some studies indicated that an increase in the physiological levels of TIMP-2 can promote MMP-2 activation and invasion in the tumor cells [31–33]. On the other hand, Zhang B *et al.* (2010) proved that in the ameloblastomas existed higher transcriptional levels of MMP-2, TIMP-2 and MMP-14 that may be implicated in the invasive biological behavior of these tumors [34]. However, recent evidence indicates that the TIMPs have additional biological effects, such as regulation of cell growth, migration and apoptosis [35, 36]. In this respect, Siqueira AS *et al.* (2010) observed in ameloblastoma a correlation between TIMP-2 and TGF-α, which probably regulate tumor proliferation [22].

An important component of tumor invasion process is the *alteration in cell–cell and cell–ECM* interactions, mediated by many kinds of cell adhesion molecules [37]. Cadherins, named for "calcium-dependent adhesion" are a class of type-1 transmembrane proteins that bound cells together within tissues. One of the members of this superfamily is *E-cadherin* a calcium-dependent cell adhesion molecule for homophilic adhesion of the epithelial cells, which maintain the epithelial tissues but also with key roles during the organogenesis and morphogenesis [38, 39]. Many authors have been reported to correlate the loss or reduction in expression of such molecules with dedifferentiation, invasion and metastasis of various neoplastic lesions.

Our investigation proved a weak or even lost E-cadherin reactivity in the keratinized areas of the acanthomatous ameloblastomas and in the granular cell clusters of the granular cell ameloblastomas. Both qualitative and quantitative the E-cadherin expression was high in stellate-reticulum like cells from the ameloblastoma follicular type. Similar results were obtained by others authors, which investigated adhesion molecules in the ameloblastoma in an attempt to understand whether these proteins play a role in the oncogenesis and cytodifferentiation of these tumors [9, 11, 40–42]. The highest E-cadherin expression in cells resembling the stellate reticulum, suggested a possible higher concentration of this protein at specific sites where it promotes the adhesion between the distant cells in this arrangement [9]. On the other hand, Kumamoto H and Ooya K (1999) related the E-cadherin expression decreasing to the periphery of neoplastic lesions from the ameloblastoma follicular type and also in the keratinized areas and granular cell clusters from the corresponding acanthomatous and granular cell ameloblastomas to the terminal differentiation of the tumor cells such as maturation and/or degenerative changes, and was not thought to reflect progression or malignant potential of the tumors [40]. Moreover, González-Alva P *et al.* (2010) said that most likely a combination of genetic, epigenetic, transcriptional, and post-transcriptional mechanisms all cooperate to weaken E-cadherin-dependent cell–cell adhesion, and thus promote invasion [11].

Epithelial–mesenchymal transition (EMT) is the process by which epithelial cells lose their polarity and cohesiveness and acquire a mesenchymal shape and increased motility [43], and takes place during the embryogenesis, tumorigenesis, metastasis, and fibrosis [44–47]. Such gain of migratory capability and autonomous cell survival underlies the development of the invasive and metastatic tumors [48]. At the basis of this process, lies the cytoskeletal intermediate filaments profile modification with the initiation of *vimentin* expression. Therefore, vimentin expression has become a canonical marker of the EMT [43].

Our investigation proved that the strongest vimentin reaction expression was observed in the stromal cells adjacent to the invasive front. In the ameloblastoma epithelial compartment, vimentin reaction was observed in the peripheral columnar/cuboidal cells from the invasive neoplastic islands, especially in those cases with bone or skin extension. Similar results have been reported by González-Alva P *et al.* (2010), which noticed that vimentin reactivity was found in the stromal cells, but partial or no reaction was observed in the neoplastic cells [11]. Therefore, they concluded that the pattern of vimentin expression in conjunction with E-cadherin reactivity would be suggestive of EMT non-involvement in the process of the ameloblastomas local invasion.

Further investigation of more cases should be carried out in an attempt to clarify the mechanisms underlying this aggressive local behavior of the ameloblastomas.

Conclusions

Our results proved that both MMP-9 and TIMP-2 are involved in the local invasion of the ameloblastoma most probable by monitoring the ECM remodeling, with the stroma playing an active role in the growth of those tumors. At the same time, E-cadherin and vimentin are involved in the control of the ameloblastic local behavior by their roles played in the EMT process. Thus, the expression of these molecules may serve as an indicator of the degree of local aggressiveness of the ameloblastic tumors.

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Corresponding author

Claudiu Mărgăritescu, Associate Professor, MD, PhD, Department of Pathology, University of Medicine and Pharmacy of Craiova, 2–4 Petru Rareş Street, 200349 Craiova, Romania; Phone +40740–152 550, e-mail: c_margaritescu2000@yahoo.com

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